P 175

COMPARISON OF CORNEAL STORAGE AT 4°C AND 31°C

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Purpose : The aim of this study is to compare the results of two series of penetrating keratoplasties, each with a different method of conservation.
Methods : The corneas of sixteen donors were conserved one of each pair at 4°C and the other at 31°C. The average duration of conservation was 3.2 days at 4°C and 13.4 days at 31°C. Average endothelial cell density evaluated with trypan blue was 3421 cells/mm^2 at 4°C. At 31°C it was 3040 cells/mm^2 before keratoplasty.
Results : Between october 1993 and february 1994, we performed 32 penetrating keratoplasties with this grafts. At 4°C, duration of follow-up was 9.2 months with a survival rate of 87.5%. Mean corneal thickness was 504 µm. Average endothelial cell count was 1659 cells/mm^2 on specular microscopy. At 31°C, duration of follow-up was 9.4 months with a survival rate of 93%. Mean corneal thickness was 507 µm. Average encothelial cell count was 1700 cells/mm^2 on specular microscopy. There is no statistical difference between corneal thickness measurement and endothelial cell count of corneas conserved at 31°C and those conserved at 4°C.
Conclusions : Our study did not reveal any difference in short outcome of penetrating keratoplasties between corneas stored at 31°C and those stored at 4°C.

P 176

CHANGES IN KERATOCYTE DENSITY OF THE HUMAN CORNEA DURING ORGAN CULTURE

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Purpose: In the cornea other cellular components than the keratocytes have been investigated extensively. It was the aim of this study to determine the normal keratocyte density in the human cornea and to describe changes in cell density during organ-culture.
Material and Methods: We investigated 92 human corneas directly post-mortem and after 15, 18, 21 and 28 days of organ-culture followed by a deswelling period of one day. After histological preparation, 6 fields in the center and another 6 in the periphery of each cornea were counted.
Results: In fresh human corneas 230 +/- 60 cells /mm2 were found in the center and 240 + /- 49 in the periphery. During organ culture the cell number decreased to a minimum after 19 days. This loss of keratocytes was predominant in the center (137+/-33 after 19 days), wheras a smaller number was lost in the periphery (172+/-46). During the following culture period the cell density increased again. After 29 days 210+/39 in the center and 184+/-37 cells /mm2 in the periphery were reached.
Conclusions: Our results confirm the rare cell countings which could be found in literature (Möller-Pedersen). A cell loss was predominant during the first three weeks of organ-culture, followed by a recreation period in the fourth week.
The higher cell loss in the center during the first three weeks can be compensated either by cell division or by migration. As the increase in the periphery after the 22th day was lower than in the center, migration may play an important role. Also ageing of keratocytes as described for the epithelium and endothelium of the cornea seems to be possible.

P 177

DOES THE ORGAN-CULTURE OF HUMAN CORNEAS LEAD TO UNIFORM CAPABILITIES OF THE STROMAL KERATOCYTES?

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Purpose: For the preservation of human donor corneas the keratocytes are becoming more and more interesting. Very few is known about this cell type, especially about the potential morphological and biochemical alterations and possible changes of growth kinetics during corneal storage.
Methods: At the time of establishing cell lines of human corneal fibroblasts in our laboratory we made some investigations with fresh and cultured human corneas. We collected the available informations concerning the donor criteria and determined the growth rate (r), cumulative cell numbers (ccn) and the cumulative population doublings (cpd) of the established cell cultures between the 3. and 16. passage for characterizing their growth behaviour. The following 3 groups were investigated: 3 cultures of fresh corneas, 3 cultures of corneas stored in MEM and 3 cultures of corneas stored in MEM and MEM with 5% Dextran 500.
Results: Donor criteria showed no influence on the success of inoculation. Pre-cultured keratocytes in MEM showed the best and homogenous results concerning r, ccn and cpd. The values for fresh corneas and corneas pre-cultured in MEM and MEM with 5% Dextran 500 were on a lower level.
Conclusions: First our results showed that donor criteria seem to have no influence on the growth behaviour of human keratocytes. Furthermore the corneas seem to become more uniform during preservation in MEM. This pre-culture seems to have a positive influence on the culturing of isolated human corneal fibroblasts.

P 178

OPTIMAL STORAGE TEMPERATURE FOR HUMAN CORNEAS IN ORGAN-CULTURE

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Introduction: The preservation of corneoscleral buttons by organ-culture at 30-32°C is an accepted storage method. Little is known however about the optimal storage temperature in a medium without dehydrating agents. Acidosis and glucose depletion are thought to be limiting factors in storage time. Therefore we studied whether slowing down corneal metabolism by lower storage temperatures could prolonge maximal storage time.
Methods: 14 Paired human corneas, discarded for transplantation because of scars in the optic centre or because of senile changes in the endothelium were used. Of every pair one cornea served as control and was stored in 60 ml EMEM medium at 31°C, while the other was placed either at 21°C or 26°C. Three ml samples were taken from the culture medium at day 7, 14, 21, 28 and 35 and used for biochemical analysis to determine the glucose and lactate concentration. pH and osmolality were measured at day 35. The corneal endothelium was morphologically evaluated after 35 days.
Results: During organ culture at 31°C the glucose concentration decreased from 5.4 mM at day 0 to zero at day 35, the lactate concentration increased from 0.0 to 7.3 mM. At 21°C and 26°C the values at day 35 were: glucose 3.0 and 1.1 mM, lactate 3.8 and 6.0 mM, respectively.
The osmolality remained constant in all cases, whereas pH values decreased from 7.47 at day 0 to 7.05 (31°C), 7.15 (26°C) and 7.43 (21°C) at day 35 respectively. No significant differences were found in endothelial morphology between 31°C and 26°C, whereas at 21°C the endothelium was found to be dystrophic after 35 days of storage.
Conclusions: Although at 21°C a sufficient amount of glucose remained in the medium and acidosis was minimal, the endothelium did not survive after 35 days. Prolongation of the storage time by reduction of the storage temperature is not a serious alternative because the temperature tolerance of the endothelium turned out to be limited.